EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON LEVELS OF RESISTIN IN GCF OF PATIENTS WITH CHRONIC PERIODONTITIS AND IN CHRONIC PERIODONTITIS PATIENTS WITH TYPE 2 DIABETES MELLITUS

Dissertation submitted to

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BRANCH – II

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This is to certify that **Dr.A.THARANI,** Post Graduate student (2015-2018) in the Department of Periodontology, K.S.R. Institute of Dental Science and Research, has done this dissertation titled "**EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON LEVELS OF RESISTIN IN GCF OF PATIENTS WITH CHRONIC PERIODONTITIS AND IN CHRONIC PERIODONTITIS PATIENTS WITH TYPE 2 DIABETES MELLITUS"** under our guidance and supervision in partial fulfillment of the regulations laid down by **The Tamilnadu Dr. M.G.R. Medical University**, Chennai – 600 032 for **M.D.S.,** (Branch –II) **Periodontology** degree examination.

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ABBREVIATIONS

ADSF	Adipocyte specific secretory factor
C	Celcius
CAL	Clinical attachment level
CRP	C reactive protein
DNA	Deoxyribonucleic acid
ECM	Extra cellular matrix
ELISA	Enzyme linked immunosorbent assay
FBS	Fasting blood sugar
FIZZ	Found in inflammatory zone
FMLF	Formyl peptide derivates
FPR	Formyl peptide receptor
GCF	Gingival crevicular fluid
GI	Gingival index
GLUT	Glucose transporters
Hb	Haemoglobin
HDL	High density lipoprotein
Ig	Immunoglobulin

IL	Interleukin
k	Kappa
LPS	Lipopolysaccharide
mm	Millimeter
MMP	Matrix metalloproteinase
NF-Kb	Nuclear factor kappa beta
ng	Nanogram
nm	Nanometer
PBMC	Peripheral blood mononuclear cells
PD	Probing depth
PDLC	Periodontal ligament cells
PI	Plaque index
PlI	Plaque index
PPD	Probing pocket depth
RELM	Resistin like molecules
RNA	Ribonucleic acid
ROR	Receptor tyrosine kinase – like orphan receptor
SNP	Single nucleotide polymorphism
SRP	Scaling and root planing

Str	Streptovidin
T2DM	Type 2 diabetes mellitus
TNF	Tumour necrosis factor
UNC	University of North Carolina
WHR	Waist hip ratio
α	Alpha
β	Beta
γ	Gamma
µl	Microlitre

INTRODUCTION

Biomarker is a characteristic, that is measured objectively and evaluated as an indicator of normal biological or pathological process and also as a pharmacologic response to therapeutic intervention. These biomarkers are useful for screening, risk assessment before diagnosis and also for selection of therapy, monitoring the therapy and recurrent diseases.[1]

Resistin is one of the newly recognised adipokine with all qualities of an ideal biomarker.[1] It was named after its characteristic function of resisting insulin. Resistin is a 12.5kDa cysteine which is a secretory protein consisting of 108 amino acids mainly involving in inflammation and found in FIZZ 3 (Found in inflammatory zone -3).[2] It is expressed in very low level in adipocytes and higher level in circulating monocytes, macrophages, lymphocytes and mononuclear leukocytes.[3] Some of the inflammatory conditions where resistin levels are found to be increased include rheumatoid arthritis, diabetes mellitus, retinopathy, chronic kidney diseases, atherosclerosis, coronary artery diseases and periodontitis.[1]

Chronic periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss and bone loss.[4]

This chronic inflammatory response in periodontitis is caused by periodontopathogens present in hard and soft deposits on the tooth surface. These microorganisms and their products (protease, collagenase, hyaluronidase, chondroitin sulfatase, endotoxins) spontaneously trigger a cascade of immunomodulatory reaction in the host, hoping to ward off the progressing infection. But what happens is, along with these cascade of events various pro inflammatory cytokines and large quantities of destructive enzymes such as matrix metalloproteinase and inflammatory mediators are produced resulting in more extracellular degradation, osteoclast activation and differentiation, leading to further collagen and bone

destruction ultimately ending up in more tissue destruction. These inflammatory mediators produced as a result of host immunomodulatory response in periodontitis serve as a biomarker for disease activity.[1,5]

Chronic periodontitis is characterised by inflammation of periodontium with the infiltration of various inflammatory cells into the periodontal tissues. These inflammatory cells are the major source for resistin. Inturn, resistin also activates NF-kB dependant cytokine release and adhesion molecule expression. Lipopolysaccharide from periodontopathogens induce resistin gene found in macrophage through a cascade involving the production of proinflammatory cytokines.[1]

As periodontitis is a sixth complication of diabetes, a bidirectional relationship has been established between diabetes and periodontitis, in which one has influence on the other. In periodontitis, with chronic subclinical inflammation, the insulin sensitivity is decreased with increase in resistin level. This increased resistin level also induces insulin resistance by influencing GLUT (Glucose Transporters) and increasing the risk for type 2 diabetes mellitus. Thus resistin could be one of the ideal biomarkers which provides link among Resistin – Periodontitis - Diabetes mellitus triad.[1]

These proteins are detected in serum, plasma and other body fluids including GCF. GCF is considered to be an important diagnostic material containing host cell products (cytokines, antibody, enzymes), products of tissues destruction, plasma-derived molecules and sub gingival microbial products. In inflammatory conditions, there will be an increase in GCF flow as it is an inflammatory exudate. Hence determination of GCF derived biomarker combined with clinical measurements provide a sensitive measure for identification of periodontal disease progression and treatment outcomes.[4]

After non-surgical periodontal therapy (scaling and root planing) the periodontal conditions heal sufficiently with decreased clinical signs of inflammation with decreased levels of resistin. This decreased levels of resistin after non surgical periodontal therapy decreases the insulin resistance ultimately increasing the insulin sensititvity. Thereby it reduces the blood glucose level in patients with diabetes mellitus. Very few studies have documented the presence of GCF resistin in chronic periodontitis, type 2 diabetes mellitus and also its level after intervention. Hence the aim of this study is to detect the levels of resistin in GCF of patients with chronic periodontitis and in patients with chronic periodontitis and type 2 diabetes mellitus and also their levels after non-surgical periodontal therapy.

AIM:

To evaluate the levels of resistin in gingival crevicular fluid (GCF) before and after non surgical periodontal therapy in systemically healthy chronic periodontitis patients and in chronic periodontitis patients with type 2 diabetes mellitus.

OBJECTIVES:

1. To evaluate the clinical parameters and levels of resistin in gingival crevicular fluid of systemically healthy chronic periodontitis patients and in chronic periodontitis patients with type 2 diabetes mellitus before non surgical periodontal therapy.
2. To evaluate the clinical parameters and levels of resistin in gingival crevicular fluid of systemically healthy chronic periodontitis patients and in chronic periodontitis patients with type 2 diabetes mellitus at 3 months following non surgical periodontal therapy.
3. To compare the levels of resistin in gingival crevicular fluid of chronic periodontitis patients with and without type 2 diabetes mellitus before non surgical periodontal therapy.
4. To compare the clinical parameters and levels of resistin in gingival crevicular fluid of chronic periodontitis patients with and without type 2 diabetes mellitus at 3 months following non surgical periodontal therapy.

Chronic periodontitis is an infectious disease resulting in inflammation within the supporting tissues of the teeth, progressive attachment loss, and bone loss. Chronic periodontitis is most common form of periodontitis, seen in adults, but can also occur in children and adolescents in response to chronic accumulation of plaque and calculus.[4]

Microbial plaque and calculus that gets accumulated on the tooth surface that is in close proximity to the gingiva is considered to be the prime etiological factor for the inflammation of periodontal tissues.[6] On the other hand, various systemic and environmental factors that affect the normal host-bacterial interaction can also accelerate the progression of periodontal diseases. It is generally a slowly progressive disease characterised by the presence of inflammation, pocket formation, attachment loss, and bone loss resulting from the direct, site-specific effects of subgingival plaque accumulation.

PATHOGENESIS OF PERIODONTAL DISEASE:

The primary etiologic agent for periodontal disease are periodontal pathogens. The periodontal microorganisms that have the ability to colonise, evade host defence mechanism, and ability to produce virulence factors are capable of causing periodontal disease. For decades, the initiation and progression of periodontitis has been considered to be caused by the presence of specific bacteria or groups of bacteria harbouring the subgingival plaque biofilm.[7]

In the current paradigm of periodontal disease, periodontal pathogens are necessary for the initiation of periodontal disease, however the extent and severity of periodontal tissue destruction are mainly dependent on the complex host-microbial interaction.[4]

Periodontal disease is characterized by a complex interplay between the subgingival biofilm and the immune mediated inflammatory events occurring in the host tissue in response to bacterial challenge.[8]In periodontitis, the primary etiological agent is specific, mainly gram negative anaerobic or facultative bacteria harbouring the subgingival biofilm. The majority of periodontal tissue destruction results from exaggerated host response to these microorganisms and their products.[7]The host immune

system gets activated in response to pathogens and produces various pro inflammatory cytokines in order to ward off the infection. These inflammatory mediators are also used as a biomarkers for the periodontal disease. Biomarkers span a broad spectrum of human health care which helps in understanding human biology, diagnosis and risk assessments of particular disease. The biomarker is either produced by a diseased organ or by the body in response to a disease.[1] Periodontitis is one such disease with various biomarkers including the newly discovered Resistin.

RESISTIN:

The novel protein, Resistin is a putative adipocyte derived signalling polypeptide which belongs to the group of adipokines. It is a 12.5kDa cysteine rich secretory protein consisting of 108 amino acids with important regulatory roles in various biological process.[5]

DISCOVERY:

Resistin was first described in 2001. It was discovered during the research on genes which are responsible for adipocyte differentiation.[9]It was also found that resistin was down regulated in mature adipocytes. The protein was named as Resistin after its proposed function of resisting insulin. It was identified originally in three different conditions. Initially it was observed in obesity and insulin resistance. Later,it was found that this protein was also involved in inflammation.[10]It is upregulated in obesity, insulin resistance and inflammation and down regulated by immunoneutralization which include insulin sensitivity.

TISSUE DISTRIBUTION AND VARIOUS CONDITIONS:

Tissue distribution of Resistin is seen in hypothalamus, pituitary, adrenal glands, pancreas, gastrointestinal tract, myocytes, spleen, white blood cells and plasma. It is also highly expressed in immune cells like monocytes, macrophages, neutrophils, lymphocytes and various body fluids in humans. It can also be detected in various inflammatory conditions like rheumatoid arthritis, diabetic

retinopathy, chronic kidney diseases, atherosclerosis, coronary heart disease and periodontitis. It is also known as FIZZ 3 (found in inflammatory -3) or ADSF (Adipocyte specific secretory factor).[1,5,9]

RESISTIN LIKE MOLECULES (RELM):

The resistin which is adipokine, is a cysteine rich protein. There are also other resistin like molecules (RELM). It includes RELM - α /FIZZ 1, RELM - β /FIZZ 2, FIZZ 3 and RELM -γ/ FIZZ 4.[1,11]

FIZZ 1/RELM- α: It is the first isolated protein in FIZZ family. It is found on chromosome 11 and located at II-q 21. It is expressed in white adipose tissue of the heart, lungs and tongue. It is present in the inflammatory zone of allergic pulmonary inflammation and is mostly found in bronchoalveolar fluid.

FIZZ 2/RELM-β: It is found in proliferating epithelium of crypt and can be detected in high levels in stool of humans.

FIZZ 3: Resistin belongs to the family of FIZZ. Cysteine is the most common amino acid in resistin which comprises approximately 12% of its amino acid sequence. It is mainly involved in inflammation and found in adipocytes and various immune cells.

FIZZ 4/RELM-γ: It is identified in hematopoietic tissues and in nasal respiratory epithelium.

STRUCTURE:

The resistin gene called as Retn is encoded with 108 amino acid polypeptide in humans and 114 aminoacid polypeptide in rats. It is secreted as disulfide – linked homodimer structure (Figure 1A,B,C). This disulphide bond has to be cleaved to intiate the bioactivity. X-ray crystallographic studies showed complex hexameric structure. It is present in two distinct assembly states.[9]

1.A high molecular mass hexamer which is more predominant.

2. A low molecular mass complex which is more bio active.[9]

FIGURE 1:Structure of resistin

FIGURE A: Monomeric structure of resistin

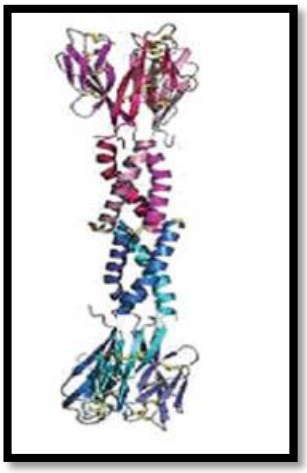


FIGURE B: Hexameric form comprising of two disulphide-linked trimers

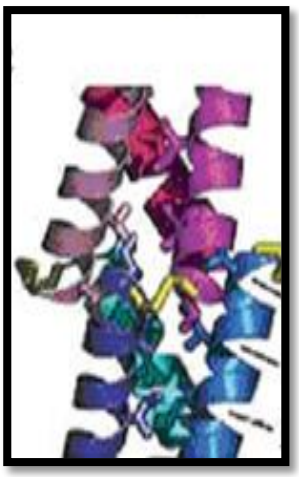
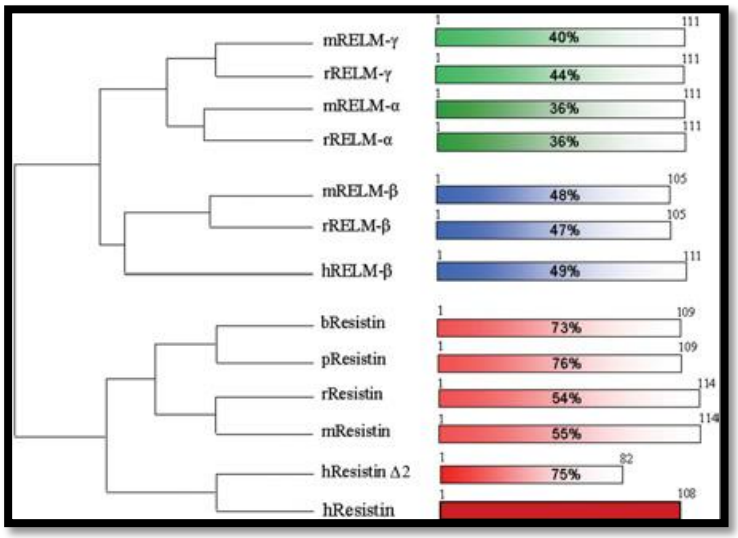


FIGURE C: Interchain disulphide linkages present in the hexameric form of resistin

FIGURE 2: Amino acid sequence identities of rodent, porcine and bovine resistin, other RELM family members and the recently identified human alternatively spliced variant of resistin, h resistin



FUNCTIONS OF RESISTIN IN INFLAMMATION:

- Resistin induces the expression of pro inflammatory cytokines, cellular adhesion molecules, chemokines and matrix metalloproteinase in PBMCs, endothelial cells and adipocytes.
- It inhibits chemotaxis and decreases the oxidative burst of human polymorphonuclear leucocytes.
- It induces osteoclast differentiation by the stimulation of nuclear factor – kB transcriptional activity.[11]

RESISTIN IN NEUTROPHILS:

Periodontopathogens present in dental plaque, biofilm and food debris gets accumulated on the tooth surface. These microorganisms and its products, endotoxins initiate a cascade of host immuno modulatory responses. This immune response of the host against pathogenic microorganisms attempts to ward off the infections. During this cascade various pro inflammatory cytokines are produced. The imbalance between these two cytokines results in over production of pro inflammatory cytokines and large quantities of destructive enzymes such as MMPs and inflammatory mediators. This leads to extracellular degradation, osteoclast activation and differentiation leading to further collagen and bone destruction.[13]

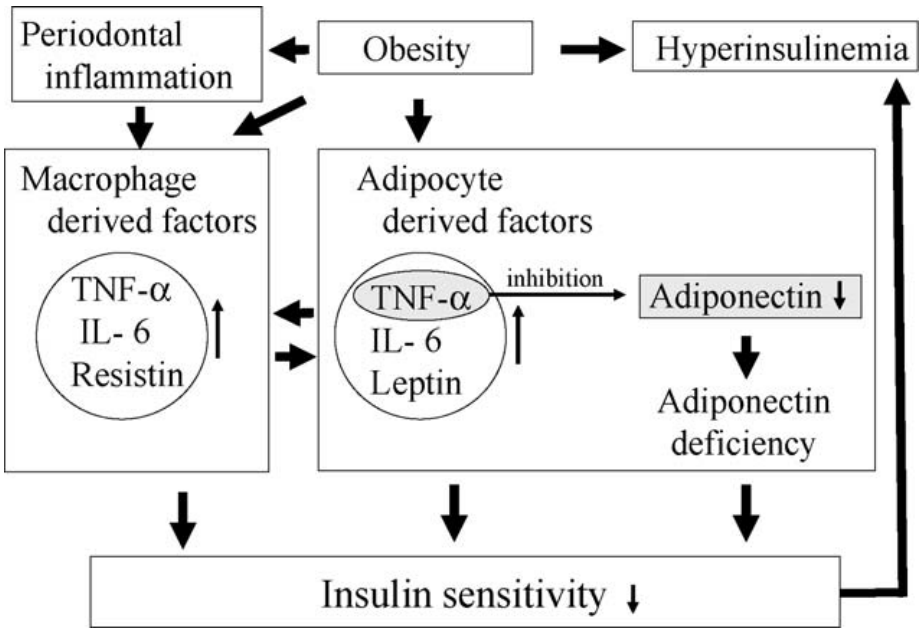
The important and major source for resistin is mononuclear leucocytes (neutrophils). The resistin is present in azurophil granules, specific granules and also on the cell membrane (membrane attached resistin) of neutrophils. The release of granular content from neutrophil is one of the major component of innate immune response. Neutrophils are one of the major fraction of leucocytes present in circulation and also at the site of inflammation. These neutrophils migrate to the site of inflammation by transendothelial migration in response to pathogens. This extravasation of neutrophils into inflamed tissue takes place by various chemoattractants.[13]

RELEASE OF RESISTIN FROM IMMUNE CELLS:

The resistin is upregulated and released from neutrophils by various pro inflammatory stimuli like TNF α, peptidoglycans, bacterial endotoxins. One of the major activator of neutrophil are these small formyl peptide derivates (FMLF) which is a chemo attractant mediator. These FMLF binds to FPR (formyl peptide receptor) and triggers the intra cellular activation mediated by G-Protein. This intracellular activation results in phosphorylation of intracellular kinases like phosphatidylinositol -3-kinase, protein C kinase, mytogen – activated protein kinases and further leads to release of granular content. The activation of neutrophil by FPR induces extracellular release of resistin also.[13]

As chronic periodontitis is a low grade infection, it is characterised by infiltration of inflammatory cells into the periodontal tissues and it acts as a source for resistin production. Inturn resistin also activates NF-kB which releases various cytokines and adhesion molecule. Lipopolysaccharides produced by periodontal pathogens also induce resistin gene in macrophage via cascade involving the production of proinflammatory cytokines.

FIGURE 3: The relationship between periodontitis and adipokines



RESISTIN-PERIODONTITIS-DIABETES MELLITUS- A TRIAD RELATIONSHIP:

Periodontitis is considered to be the sixth complication of diabetes and a two way relationship has been established between diabetes and periodontitis. In periodontitis with chronic subclinical inflammation, the insulin sensitivity is decreased with increase in resistin level. Resistin plays an important role in insulin resistance, thus increasing the risk for type 2 diabetes mellitus. The insulin resistance is mediated by the action of resistin on the expression and localisation of GLUT (Glucose

transmitter). It was found that chronic exposure to resistin adversely affects the entry of glucose into cells and thus counteracting the effect of insulin. Over expression of receptor tyrosine kinase – like orphan receptor (ROR 1) induces the expression of GLUT 1 and GLUT 4 in pre adipocytes, in which this action can be reversed by resistin. The interaction of the resistin and extracellular ROR 1 receptor results in ROR 1 phosphorylation and regulates the suppression of cytokine signalling, GLUT 1 and GLUT 4 expression. ROR 1 plays an important role in adipogenesis and glucose homeostasis.[1]The amount and severity of periodontal tissue breakdown is influenced by T2DM.[14]

GINGIVAL CREVICULAR FLUID:

These proteins are detected in serum, plasma and other body fluids including GCF.GCF is considered to be an important diagnostic aid containing host cell products (cytokines, antibody, enzymes), products of tissues destruction, plasma-derived molecules and sub gingival microbial products. Gingival crevicular fluid is a transudate in healthy gingival crevice and an exudate in the inflammatory conditions mainly in periodontitis. It contains saccharides, electrolytes, cellular components, decayed products of periodontal tissue, bacteria and their products, various proteins including serum proteins, immunoglobulins, enzymes, periodontal tissue derived proteins and inflammation derived proteins, cytokines and bacterial proteins. Resistin is one of the proteins present in GCF. The increased levels of interleukin 1β, interleukin 6, TNF α which are pro inflammatory cytokines, are present in GCF of periodontitis patients. Hence determination of GCF derived biomarker combined with clinical measurements provide a sensitive measure for identification of periodontal disease progression.[4,15]

REVIEW OF LITERATURE

Hasegawa et al.(2005)[16] correlated the relationship between serum resistin levels in type 2 diabetes mellitus patients and the patients with adiposity. About 111 type 2 diabetes patients and 98 non-diabetic patients were included in this study. Blood samples were collected from all patients with overnight fast. Serum resistin level was determined by ELISA. The results showed significant increase in serum resistin level in type 2 diabetes mellitus patients when compared with non-diabetic patients. From this study it was concluded that presence of diabetes mellitus and High density lipid (HDL) cholesterol were significant predictors of serum resistin levels.

Bo S et al. (2005)[17] evaluated the association between serum resistin levels, insulin resistance and marker of inflammation (C reactive protein). A total of 300 randomly selected patients with normal BMI were included. From all the patients venous blood was collected after an overnight fast. Glucose level, HDL cholesterol, uric acid, triglyceride, insulin, nitroglycerine and resistin were evaluated. Serum resistin level was evaluated by ELISA. The results showed that resistin, CRP level were significant in over weight patients. Resistin level was associated with CRP levels in all patients. From this study it was concluded that serum resistin was directly correlated with CRP and it is secreted in response to chronic low grade inflammation.

Furugen R et al. (2008)[18] conducted a study to clarify the relationship between serum adipokine level in patients with and without periodontitis. About 158 elderly Japanese people with and without periodontitis were included in the study. The clinical parameters includes probing pocket depth of ≥6mm in atleast one teeth for periodontitis and ≤6mm for control group. Bleeding on probing in ≥10% sites for periodontis patients and <10% for control group.BMI and FBS were analysed. Blood sample s were collected and stored at -80ºC.The levels of adiponectin, resistin, TNF α and IL-6 were examined using ELISA. Results showed higher levels of resistin in periodontitis patients than in control patients. No significant difference were observed in

adiponectin, IL-6 and TNF α levels between subjects with and without periodontitis. Serum adiponectin tended to decrease in patients with periodontitis but not significantly.

Saito et al. (2008)[19] conducted a study to correlate the relationship between serum levels of adipokine in periodontally healthy and chronic periodontitis patients. A total of 76 patients were included in the study with 2 groups. Group 1 consists of 34 patients with moderate to severe periodontitis and Group 2 consists of 42 healthy subjects. Periodontitis patients were categorized by probing depth ≥6mm in atleast 1 teeth and clinical attachment level in atleast 3 teeth with≥4mm.The subjects with healthy gingiva should have probing depth <3mm and no more than 2 teeth that bleed while probing. Fasted blood samples were collected and stored in freezer. Resistin, adiponectin, TNF α and IL-6 were analysed using ELISA. Statistical analysis was done. The results showed higher levels of adipokines and resistin in periodontitis patients than with healthy patients. Adiponectin levels were reduced in periodontitis patients but they were not statistically significant. From this study it was concluded that serum resistin level is associated with periodontitis and it may also have triangular relationship between obesity, type 2 diabetes mellitus and periodontal disease.

Gharibeh et al. (2010)[20] evaluated the insulin resistance markers in type 2 diabetes Mellitus patients, obesity and plasma resistin levels. Study includes 140 diabetic patients and 125 non-diabetic patients. BMI was calculated. Blood were obtained in morning for all the patients after overnight fast. Human resistant in plasma was measured by ELISA. Results showed higher levels of resistin in diabetic patients than non-diabetic individuals. The patients who were > 60 years of age showed significant resistin levels than the patients who were below 40 years of age. It was concluded that resistin was involved in increased adiposity, insulin resistance and type 2 diabetes mellitus.

Devanoorkar et al. (2012)[21] conducted a study to determine the serum resistin level in periodontal health and disease patient and also evaluated the resistin level after non surgical

periodontal therapy. A total of 40 patients with two groups were included. Group I includes 20 healthy patients with probing depth ≤ 3mm , clinical attachment level ≤ 1mm, Full mouth bleeding score less than 1. Group II- included 20 chronic periodontitis with 5mm pocket depth, clinical attachment level ≥2mm in at least four teeth with radiographic evidence of bone loss. Other parameters like gingival index, bleeding index, BMI were evaluated. About 2ml of blood samples were collected and centrifuged following this non surgical periodontal therapy. Blood samples were collected from group II patients two weeks after therapy. After analysis using ELISA, results showed higher serum levels in chronic periodontitis patients when compared to healthy patients. This study concluded that serum resistin levels does not show any difference between group I and group II and also decrease in serum resistin level following non surgical periodontal therapy.

Hiroshima et al. (2012)[12] evaluated the GCF resistin level in patients with and without periodontitis and Type II Diabetes mellitus and also investigated the release of resistin from neutrophils after induction by Porphyromonas gingivalis lipopolysaccharides. The study includes 63 patients and were divided into 3 groups. It consist of 24 chronic periodontitis patients, 18 Diabetes mellitus related periodontitis patients, 21 healthy patients. Periodontitis was defined by the probing depth of ≥4mm and for Diabetes patients HbA1c >5.8%. GCF was collected using paper strips and measured using Periotron 8000.The assay was done by western blotting and ELISA. Invitro analysis was done for neutrophils. Blood samples were collected from 6 healthy volunteers and the neutrophils were separated from the whole blood and seeded in culture dishes. The incubation is done with and without P-LPS. After culture, cells and medium were separately collected, centrifuged and used for analysis by ELISA. The results showed higher levels of resistin in periodontitis and Diabetes mellitus patients. P-LPS also increases resistin release from neutrophils which was also decreased in the presence of polymerisation inhibitors. From this study it was concluded that GCF resistin level were increased in periodontitis patients and is related to P-LPS induced release from neutrophils.

Kido et al. (2012)[15] analysed the protein components in gingival crevicular fluid of healthy and periodontitis patients using mass spectrometry. This study includes 8 periodontitis patients with ≥ 6mm and 1 periodontally healthy patient with ≤ 2mm.About 10µl of GCF was collected using paper strips and gel digestion was done. The samples were electrophorectically separated and analysed by nano liquid chromatography and mass spectrometery. The peptides detected were confirmed by western blotting. Results showed that 104 proteins were detected in both healthy and periodontitis patients. One among them is resistin. From this study it was concluded that multiple protein components were detected in gingival cervicular fluid and resistin was one among them.

Patel et al. (2013)[22] determined the levels of resistin in serum and gingival crevicular fluid and compared its level in patients with and without periodontitis and type 2 diabetes mellitus patients and correlated it with single nucleotide polymorphism at -420. The study includes a total of 96 patients and were divided into four groups.Group1 includes 24 healthy patient, Group 2 includes 24 periodontitis patients with uncontrolled diabetes,Group3 includes24 periodontitis patient with controlled diabetics and Group4 includes 24 chronic periodontitis patients without diabetes mellitus. The categorisation of groups was done based on the clinical parameters-Gingival index, clinical attachment level, probing pocket depth, haemoglobin A1c.GCF was collected on the next day after clinical examination using microcapillary pipette only for about 10mins to prevent traumatism. The collected GCF sample was stored at -70ºC.About 4ml blood was collected, centrifuged at 3000gm for 5min,serum was separated, transferred to plastic vial and stored at -70ºC. The samples were analyzed using ELISA.SNP-420 typing was done by DNA probes. Results after statistical analysis showed higher levels of resistin in both serum and GCF sample in all patients except the healthy subjects. GG genotype at-420 was significantly associated with periodontal inflammation and resistin level. This study concluded that resistin levels were higher in periodontitis and diabetes patients and SNP-420 is associated with periodontal inflammation and resistin levels.

Zimmermann et al. (2013)[23] conducted a study to evaluate the circulating levels of adipokines in obese patients with and without periodontitis. A total of 78 patients were included in the study with 4 groups. Group 1 includes 20 normal weight patients without periodontitis, Group 2 includes 20 normal weight patients with periodontitis, Group 3 includes 18 obese patients without periodontitis and Group 4 includes 20 obese patients with periodontitis. The clinical parameters like bleeding on probing, plaque index, probing depth and clinical attachment level were evaluated in all six sites. In addition to this anthropometric measurements like weight, height, waist and hip circumferences were measured to calculate BMI and waist-hip ratio to categorize into obese and normal weight patients.GCF sample was collected using paper strips and placed in microcentrifuge tube and stored at -80ºC. Fasted blood samples were collected, centrifuged, serum was separated by centrifugation and stored at -80ºC. The analysis was done using ELISA. The result shows higher levels of resistin in periodontitis group than in non periodontitis group. The group with obese patients showed higher TNF α levels. Serum IL-6 were highly correlated in patients who are obese with chronic periodontitis. The study concludes that resistin is present in both periodontitis and obese patients which favours proinflammation.

Gokhale et al. (2014)[2] evaluated the levels of resistin in GCF of healthy, periodontitis and type 2 diabetes mellitus patients. A total of 60 patients were included in the study with 4 groups. Each group consists of 15 patients. Group 1 included 15 healthy patients with GI score ≤1 without loss of attachment, Group 2 includes systemically healthy chronic periodontitis patients with GI score≥1 and probing depth ≥5, Group 3 includes patients with type 2 diabetes mellitus with GI score ≤1 without any loss of attachment and with HbA1c ≥6.5%, Group 4 includes chronic periodontitis patients with type 2 diabetes mellitus with GI score ≥1,probing depth ≥5, HbA1c ≥6.5%. The clinical parameters - plaque index, gingival index, probing depth were evaluated.GCF collection is done in tooth with greatest probing depth. Supragingival plaque is removed with curettes, air dried, isolated with cotton rolls and microcapillary pipette is placed at the entrance of

the sulcus. About 4 microlitre of GCF is collected and stored at -20ºC. The evaluation of resistin from GCF is analysed by ELISA. The results showed highest levels of GCF in patients with chronic periodontitis with type 2 diabetes mellitus and lowest levels were found in healthy patients. From this study, it was concluded that GCF resistin levels were increased in chronic periodontitis patients with type 2 diabetes mellitus. Hence GCF resistin can be used as an inflammatory biomarker.

Goncalves et al. (2015)[24] conducted a study to evaluate the effects of non surgical periodontal therapy on serum and GCF levels of adipokines in patients with chronic periodontitis with or without obesity. Study consists of 40 patients with two groups. Group1 involves 20 patients who were obese with periodontitis. Group 2 includes 20 patients who were non obese with periodontitis. The chronic periodontitis patients were grouped based on the clinical parameters like > 30% of teeth with probing depth and clinical attachment level ≥ 4mm and in atleast one site with probing depth and clinical attachment ≥5mm. Other parameters includes HbA1c <6.5%,FBS 70 to 90 mg/dl and CRP<6mg/l. Anthropometric measurements like height, weight, waist and hip circumference were measured to calculate BMI and WHR calculation to categorise obese patients. Nonsurgical periodontal therapy (scaling and root planning was done within two weeks). GCF samples were collected from shallow and deep pockets using paper strips and stored at -80ºC. Fastened blood samples were collected, centrifuged and stored at -80ºC.Clinical parameters, GCF and blood samples were collected at baseline,3,6and 12 months post therapy analysis was done by ELISA. A total of 4 GCF samples and one serum sample were analysed for each patient. The results showed higher levels of resistin and TNF α in shallow sites in obese patients than in non obese at 6 and 12 after therapy. IL 6 was higher in deep sites. Periodontal therapy yielded statistically improvement in clinical parameters. It was concluded that obesity has an influence on periodontal levels of adipokines independent of periodontal therapy and also SRP did not affect the levels of adipokines in all patients.

Kang S-K et al. (2015)[25] conducted an invitro study to evaluate the effects of nicotine and LPS on expression of resistin and the effect of expressed resistin on inflammatory cytokines, ECM, MMPs in human periodontal ligament cells. LPS from Porphyromonas gingivalis and nicotine were collected. IGg was obtained from rabbit polyclonal antiserum. Interfering RNAs against resistin were purchased. PDLCs were obtained from premolar and cultured. Upon stimulation with inflammatory human promoters, human PBLCs produced proinflammatory cytokines and chemokines . After culturing they were treated with LPS for 24 hours. The cells were collected by centrifugation and pretreated with resistin inhibitor Iso, resistin specific si RNA. The final measurement was done using ELISA. The results showed upregulation of resistin mRNA expression and its production in PDLC. LPS and nicotine mediated the expression of TNF α, IL-1β, IL-6, IL-12 and MMPs. Pretreatment with resistin inhibitor blocked nicotine LPS induced activation of various factors. From this study it was concluded that inhibition of resistin has anti-inflammatory effect which decreased the levels of inhibitory cytokines thus preventing the ECM breakdown.

Mittal et al. (2015)[26] observed the correlation between periodontitis and rheumatoid arthritis by using GCF resistin as a potent inflammatory marker. The study includes 100 patients with 4 groups. Group A consists of healthy individuals with with PI and GI <1 without any probing depth ≥5mm. Group B includes systemically healthy chronic periodontitis patients with PI and GI >1, PD≥5mm with radiographic evidence of boneloss. Group C includes rheumatoid arthritis patients without chronic periodontitis. Group D includes patients with periodontitis and rheumatoid arthritis. GCF collection was done by micropipettes with deepest periodontal pockets -20ºC. Results showed that highest level of resistin was observed in patients with chronic periodontitis and rheumatoid arthritis and lowest level of resistin in healthy individuals. GCF resistin levels were also correlated with PI, GI and probing depth. From this study it was concluded that GCF resistin levels were increased in both chronic periodontitis patients and it can be used as a potent inflammatory biomarker.

Rao et al. (2016)[27] determined the serum and salivary levels of resistin in obese patients with chronic periodontitis. The study includes 30 patients and were divided into 2 groups. Group I includes obese individuals with healthy gingiva and Group II includes obese individuals with chronic individuals. Inclusion criteria includes the presence of atleast 20 teeth and healthy gingiva with probing depth of ≤ 3mm and for periodontitis >30% with clinical attachment level of >3mm.Clinical parameters include gingival index, clinical attachment level, BMI and obesity. All the clinical measurements were determined. After this serum and saliva sample collection was done. Unstimulated whole saliva and blood samples were collected. Analysis was done using ELISA. Statistical analysis was done. The results showed highest resistin levels in serum and salivary sample of periodontitis patients. Correlation of serum resistin concentrations with salivary resistin concentration was positive among both the groups. A positive correlation was observed between resistin and clinical parameters which was statistically significant. From this study it was concluded that resistin can be considered as a potential biomarker for periodontal diseases and obesity. This was also found to play a role in inflammatory diseases.

Suresh et al. (2016)[28] correlated the GCF resistin levels in obese patients with and without periodontitis and also correlated the resistin levels with disease severity. The study includes a total of 90 patients with 4 groups. Group 1 includes 20 obese patients with generalised chronic periodontitis , Group 2 includes 25 obese patients with healthy periodontium, Group 3 includes 25 non obese subjects with generalised chronic periodontitis and Group 4 includes 15 non obese subjects with healthy periodontium. Healthy patients were included based on GI score – 0 and generalised chronic periodontitis were included by clinical attachment level of 3-5mm at more than 30% of sites. Other clinical parameters like plaque index and probing depth were also measured. Obese patients were categorized based on BMI >25kg/m^2. GCF collection was done in multiple sites for healthy patients using micropipettes to get a volume of 2 microlitre and then stored at -71ºC. Analysis was done using ELISA. This study concluded that GCF resistin levels were

significantly higher in obese patients with generalised chronic periodontitis than with non obese subjects with healthy periodontium.

Longo et al. (2017)[29] evaluated the interferences of GCF derived inflammatory markers from periodontitis patients on glycemic control and type 2 diabetes mellitus. A total of 21 subjects were included in the study among which 14 were diabetic and 7 were non diabetic patients with chronic periodontitis in all 21 subjects. The inclusion criteria showed probing depth and clinical attachment level in ≥30% sites with ≥4mm and bleeding on probing. Previously diagnosed diabetes mellitus before 3 years and under medication with HbA1c with ≥8.0% and controlled diabetes as <8% and non diabetic subjects as <6%. Clinical parameters like plaque index, gingival index, probing depth and attachment level measured with UNC probe. GCF sample was collected using paper strips in all teeth with probing depth >6mm and stored at -80ºC.Analysis for cytokines was done by ELISA. The results showed high levels of inflammatory cytokines from the samples collected from deep periodontal pockets. There was no statistical difference between IL-6, IL-8,TNF α according to the presence of diabetes and HbA1c levels. From this study it was concluded that inflammatory mediators in GCF are dependant to the local response and doesnot correlate with diabetic status.

MATERIALS AND METHODS

PATIENT SELECTION:

The study includes a total of 37 patients with 20 systemically healthy chronic periodontitis patients and 17 chronic periodontitis patients with type 2 diabetes mellitus.The patients were selected from the outpatient ward who visited the Department of Periodontology, KSR Institute of Dental Science and Research, Tiruchengode, Tamilnadu. The study protocol was analys ed and approved by the Institutional ethical committee and review board. Study protocol was explained to the patients and written informed consent was obtained before enrollement.

SELECTION CRITERIA:

INCLUSION CRITERIA:

For Group I: (Systemically healthy Chronic periodontitis patients)

- Systemically healthy individuals
- GI Score > 1
- Minimum of 3 teeth with PD ≥ 5 mm
- RBS Level ≤ 120 mg/dl

For Group II: (Chronic periodontitis patients with type 2 diabetes mellitus)

- Patients with type 2 diabetes mellitus
- GI Score > 1
- Minimum of 3 teeth with PD ≥ 5 mm
- RBS Level > 200 mg/dl

EXCLUSION CRITERIA:

- Patients with systemic disorders.
- Patients who received previous periodontal treatment.
- Patients with antibiotic / anti-inflammatory drug regimen prior to the study.
- Patients who are smokers or use of smokeless tobacco in any form.
- Pregnant or lactating women.

STUDY DESIGN:

The study includes a total of 40 patients with two groups – 20 patients in each group.

GROUP I : Systemically healthy chronic periodontitis patients

GROUP II: Chronic periodontitis patients with type 2 diabetes mellitus

The clinical parameters including plaque index (PlI), gingival index (GI), probing depth (PD) and clinical attachment level (CAL) were recorded for all the patients at baseline and at 3 months following non surgical periodontal therapy. GCF samples were collected from the tooth with deepest periodontal pocket at baseline and at 3 months following non surgical periodontal therapy. The levels of resistin in GCF samples were analysed using an Enzyme Linked Immunosorbent Assay.

Armamentarium for invivo examination:

1. Sterile surgical gloves
2. Face mask
3. Mouth mirror
4. William's calibrated periodontal probe

5. Explorer
6. Stainless steel tray
7. Cotton pliers
8. Sterile cotton pellets and gauze

Armamentarium for GCF collection

1. Micro capillary pipette
2. Pipette
3. Plastic vials
4. Phosphate buffer solution
5. Thermocol box
6. Dry ice.

Armamentarium for nonsurgical periodontal treatment

1. Ultrasonic scaler and tips
2. Curettes
3. Local anaesthetics (2% lignocaine with 1:80,000 adrenaline)

Armamentarium for assay procedure

1. ELISA kit
2. ELISA reagent
3. ELISA reader

Clinical parameters:

1. Plaque index- Loe's modification (1967)[30]
2. Gingival index- Loe's modification (1967)[30]
3. Probing pocket depth[4]
4. Clinical attachment level[4]

PLAQUE INDEX (PlI)- LOE'S MODIFICATION (1967)

The Plaque Index was described by Silness.J and Loe.Hin 1964 and modified by Loe.H in 1967. The plaque was assessed after air drying the teeth on four sites namely mesiobuccal, midbuccal, distobuccal and lingual.[30]

Instrument used:

1. Mouth mirror
2. Dental explorer

The tooth were air-dried and examined visually. When no plaque is visible, an explorer was used on the surface. The explorer was passed across the surface in the cervical third and near the entrance to the gingival sulcus. The following scores are given.[30]

Scores for Plaque Index

Score	Criteria
0	No plaque
1	A film of plaque adhering to the free gingival margin and adjacent area of the tooth. The plaque may be seen only by running a probe, across the tooth surface.
2	Moderate accumulation of soft deposits within the gingival pocket, on the gingival margin and/or adjacent tooth surface, which can be seen by the naked eye.
3	Abundance of soft matter within the gingival pocket and /or on the gingival margin and adjacent tooth surface.

Calculation of Plaque index:

Plaque score for the area:

Each site (disto-facial, facial, mesio-facial, lingual) is assigned a score from 0 to 3

Plaque score for a tooth:

The scores from the four sites of the tooth are added and then divided by four.

Plaque score for the individual:

The indices for each of the teeth are added and then divided by the total number of teeth examined. The scores ranged from 0 – 3.[30]

Interpretation:

Excellent	0
Good	0.1 – 0.9
Fair	1.0 – 1.9
Poor	2.0 – 3.0

GINGIVAL INDEX – LOE'S MODIFICATION (1967)

The Gingival Index (GI) was developed by Loe.H and Silness.J in 1963, solely for the purpose of assessing the severity of gingivitis and its location in four possible areas by examining only the qualitative changes (i.e., severity of the lesion) of the gingival soft tissue. In 1967, Loe.H detailed the sequence of examination to include entire teeth instead of six teeth.[30]

Instruments used:

1. Mouth mirror
2. Periodontal probe.

The tissues surrounding each tooth were divided into four gingival scoring units: distofacial papilla, facial margin, mesio-facial papilla and the entire lingual gingival margin. The teeth and gingiva should be dried lightly with a blast of air and /or cotton rolls. Each of the 4 gingival units were assessed and following scores were given.[30]

Gingival index score

Score	Criteria
0	Absence of inflammation/normal gingiva.
1	Mild inflammation, slight change in color, slight edema; no bleeding on probing.
2	Moderate inflammation; moderate glazing, redness, edema and hypertrophy, bleeding on probing.
3	Severe inflammation; marked redness and hypertrophy, ulceration, tendency to spontaneous bleeding.

Calculation of Gingival Index:

Gingival Index Score for the site:

Each site (disto-facial, facial, mesio-facial, lingual) is assigned a score from 0 to 3.

Gingival Index Score for a tooth:

The scores from the four areas of the tooth are added and then divided by four.

Gingival Index for the individual:

The indices for each of the teeth are added and then divided by the total number of teeth examined. The scores ranged from 0 – 3.[30]

Interpretation:

Gingival scores	Condition
0.1 – 1.0	Mild Gingivitis
1.1 – 2.0	Moderate Gingivitis
2.1 – 3.0	Severe Gingivitis

PROBING POCKET DEPTH (PPD) AND CLINICAL ATTACHMENT LEVEL (CAL):

Probing pocket depth was measured as the distance between free gingival margin and the base of the pocket and clinical attachment level was measured as the distance between the cemento enamel junction and the base of the pocket.[4]

GCF COLLECTION:

GCF samples were collected from all patients from the tooth with deepest periodontal pockets at baseline at 3 months following nonsurgical periodontal treatment.The selected tooth was dried

using a three- way air syringe. After supragingival plaque was removed using sterile curets, the sites were isolated using cotton rolls to prevent contamination with saliva. GCF collection was done using a calibrated volumetric microcapillary pipette which has markings from 1µl to 5µl. A volume of 4µl of GCF was collected by gently placing the sterile microcapillary pippette at the entrance of the gingival sulcus.GCF collection can be done for 10 – 15 minutes.[2,31] If GCF was not collected in allotted time, the site and GCF can be discarded. This was done to prevent trauma to gingiva. Microcapillary pipettes contaminated with blood were discarded. The collected GCF was then transferred to eppendorf tube and the sample was diluted in Phosphate Buffer Saline (PBS) to make a total volume of 120µl.These air tight eppendorf tubes were placed immediately in thermocol box containing dry ice as atransporting medium and stored at-80˚C.

NON-SURGICAL PERIODONTAL THERAPY:

All the patients received non-surgical periodontal therapy, which included intensive oral hygiene phase, full-mouth scaling and root planing, maintenance and monitoring of oral hygiene. During the first visit (at baseline) complete scaling using ultrasonic scaler was performed and root planing was performed using curettes until a smooth, hard and calculus-free root surface was achieved. Oral hygiene instructions were given to all the subjects. Clinical parameters were recorded and GCF samples are obtained at baseline (prior to SRP) and 3 months following the therapy.

ASSAY PROCEDURE:

The Resistin levels in the collected GCF samples were measured using Enzyme Linked Immunosorbent Assay (ELISA). The assay employed the quantitative sandwich ELISA technique.

ELISA is one of the immunoassay method using antibodies to capture an antigen and an enzyme labelled antibody to estimate the amount of antigen. The desired antigen is captured by one antibody and bound to the plate. A second antibody binds the immobilized antigen for detection and

quantification. The antigen must contain at least two non-overlapping epitope sites capable of binding different antibodies.

Human Resistin ELISA kit from Bioassay technology laboratory was used for the assay. The assay procedure was done based on the manufacturers instruction.

INSTRUMENTS AND MATERIALS USED TO ASSESS RESISTIN IN GCF:

1 Standard(6400ng/L) 0.5ml

2 Standard diluent 3ml

3 MicroELISA Stripplate 12w×8s

4 Str- HRP-Conjugate Reagent 6ml

5 30×wash solution 20ml

6 Biotin-RESISTIN Ab 1ml

7 Chromogen Solution A 6ml

8 Chromogen Solution B 6ml

9 Stop Solution 6ml

10 Closure plate membrane 2

11 Sealed bag 1

12 37℃incubator

13 Standard Enzyme reader.

14 Precision pipettes and Disposable pipette tips

15 Distilled water

16 Disposable tubes for sample dilution

17 Absorbent paper

TEST PRINCIPLE:

The kit uses a double-antibody sandwich enzyme-linked immunosorbentassay (ELISA) to assay the level of Human Resistin（RESISTIN）in samples. Add Resistin（RESISTIN） to monoclonal antibody enzyme well which is pre-coated with Human Resistin（RESISTIN）monoclonal antibody, incubated and then add Resistin（RESISTIN) antibodies labeled with biotin, and combined with Streptavidin-HRP to form immune complex; then carry out incubation and washing again to remove the uncombined enzyme. Then add Chromogen Solution A, B, the color of the liquid changes into the blue, And at the effect of acid, the color finally becomes yellow. The chroma of color and the concentration of the Human substance Resistin（RESISTIN）of sample were positively correlated.

STANDARD PREPARATION:

Five clear test tubes were numbered from S1 to S5.

STANDARD NO 5 : Pipette 120 µl of original standard reagent and transfer to test tube S5.Add 120 µl of standard diluents.

STANDARD NO 4: Pipette 120 µl of standard No 5 and transfer to tube S4.Add 120 µl of standard diluents.

STANDARD NO 3: Pipette 120 µl of standard No 4 and transfer to tube S3.Add 120 µl of standard diluents

STANDARD NO 2: Pipette 120 µl of standard No 3 and transfer to tube S2.Add 120 µl of standard diluents

STANDARD NO 1: Pipette 120 µl of standard No 2 and transfer to tube S1.Add 120 µl of standard diluents

FIGURE 4:SAMPLE DILUTION

ASSAY PROCEDURE:

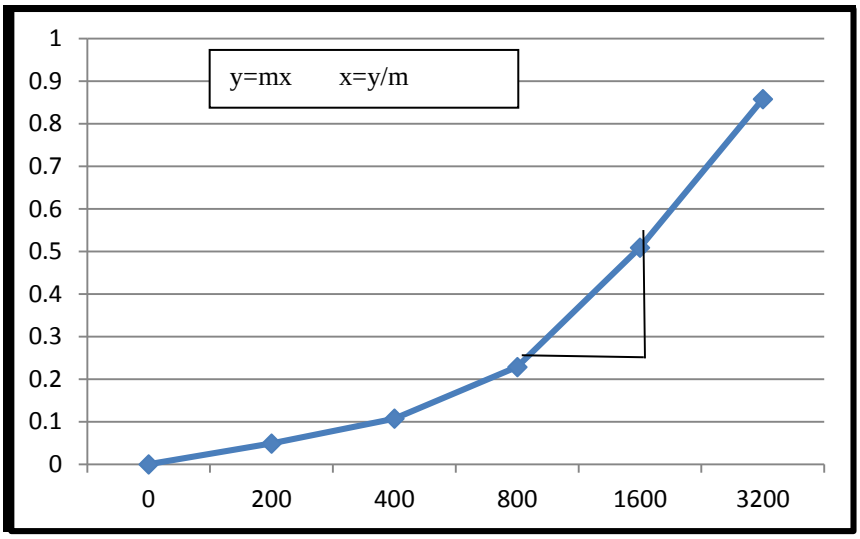
1. Duplication of blank,standard and sample were done.
2. **Blank 1**: Blank 1 is prepared by adding chromogen A 50 µl, chromogen B 50 µl, stop solution 50 µl and distilled water 100 µl.
3. **Blank 2**: Blank 2 is prepared by adding chromogen A 50 µl, chromogen B 50 µl, stop solution 50 µl , streptavidin HRP 50 µl and distilled water 50µl.
4. **Sample**: About 40 µl of sample is taken to which 10 µl of resistin antibody and 50 µl of streptavidin 50 µl is added. It is sealed with membrane and is gently shaked. Incubation is done for 60 minutes at 37ºC.
5. 30x washing concentrate is diluted 30 times with distilled water and is used for manual washing.
6. Washing: After incubation, remove the membrane carefully.The remaining liquid in enzyme plates are removed.0.35ml of washing solution is added and marinated for 1-2 minutes. The excess liquid is drained. The process was repeated 5 times.
7. Add 50 µl of Chromogen A and 50 µl of Chromogen B to the sample.
8. Incubation is done at 37ºC for 10 minutes.

9. After 10 minutes 50µl of stop solution was added to stop the reaction.(colour changes from blue to yellow colour)
10. The plate is read at wavelength of 450nm using ELISA reader. The absorbance was checked periodically.

CALCULATION OF RESULTS:

The standard curve was plotted on X-Y graph paper, with standard concentration on the x-axis and absorbance on y-axis. The best- fit straight line was drawn through the standard points. Resistin concentrations were assessed by comparing the average sample optical density readings to the concentrations from the assay standard curve.

CHART 1: X-Y GRAPH



SENSITIVITY:

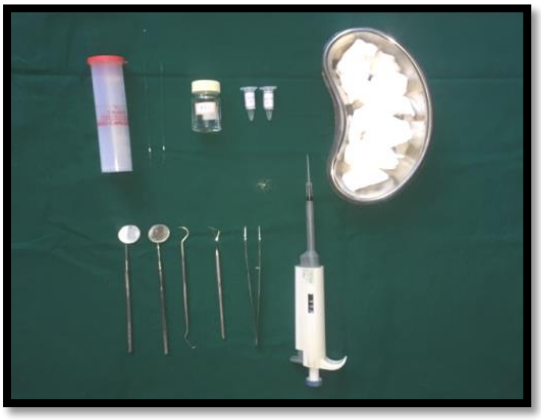
The minimum detectable dose of Human Resistin was determined to be 10.21ng/L. Minimum detectable dose is defined as the analyte concentration resulting in an absorbance that is 2 standard deviations higher than that of the blank (diluents buffer).

SPECIFICITY:

This ELISA antibody pair detects Human Resistin. Other species were not determine

FIGURE 5 : ARMAMENTARIUM

A. Armamentarium for clinical examination



B. Armamentarium for GCF collection

C. Armamentarium for non surgical periodontal therapy

FIGURE 6 : PRE-OPERATIVE PHOTOS OF CHRONIC PERIODONTITIS PATIENT

A. Front view

B. Lateral view

C. Measuring probing depth

FIGURE 7: GCF COLLECTION

A. Transferring PBS into eppendorf tube

B.GCF collection using microcapillary pipette

C. Transferring GCF sample into eppendorf tube

D. Storing the sample in dry ice

E. Deep freezer for storage of samples

FIGURE 8: NON SURGICAL PERIODONTAL THERAPY

A. Scaling using ultrasonic scalers

B. Root planing done using curettes

FIGURE 9 :POST OPERATIVE PHOTO

FIGURE 10 : ELISA PLATE WITH 96 WELLS

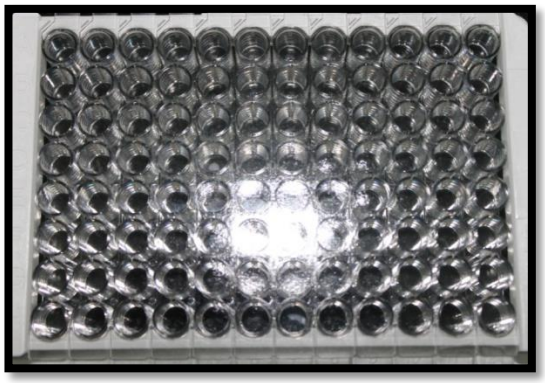


FIGURE 11 : ELISA REAGENTS

FIGURE 12: GCF SAMPLE FOR ASSAY

FIGURE 13 : STANDARD PREPARATION

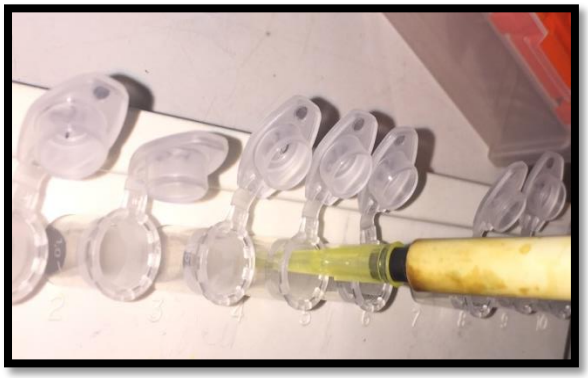


FIGURE 14 : ELISA PROCESSING

FIGURE 15 : AFTER ADDING CHROMOGEN

FIGURE 16: ADDING STOP SOLUTION

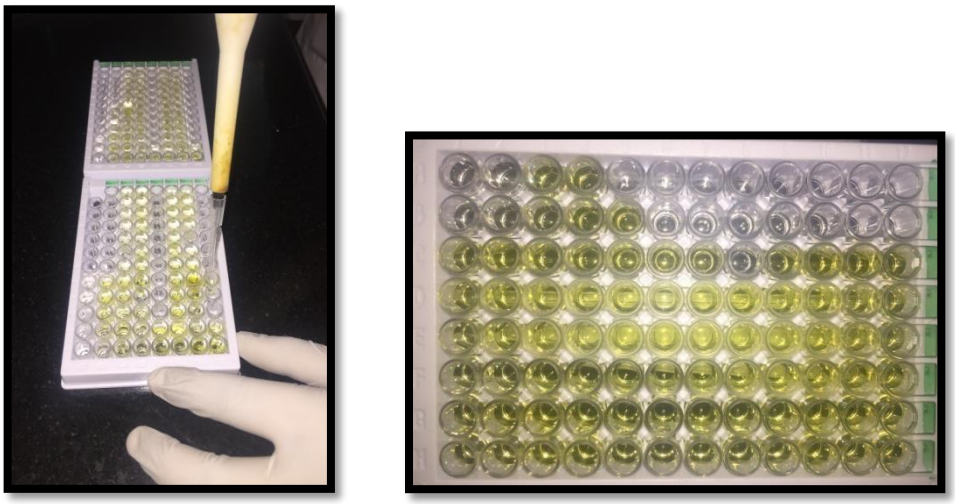
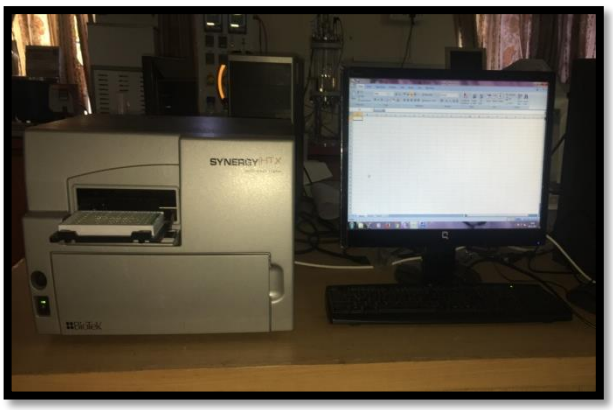


FIGURE 17: ELISA READER



Data were analyzed using the statistical software SPSS 16 (SPSS, Chicago, IL, USA). Shapiro wilks test was used to assess normality of the obtained data. Descriptive statistics was obtained. Based on distribution of data comparisons between the study groups, the analysis was performed using the Mann whitney U test (non normal distribution). Wilcoxon signed rank test (Intragroup analyses) was performed to identify the changes in clinical parameters before and after treatment in study groups. $P < 0.05$ was accepted as statistically significant.

P VALUE:

The P value or calculated probability is the estimated probability of rejecting the null hypothesis of a study question when that hypothesis is true. Differences between the two populations were considered significant when $p < 0.05$.

Table No 1, Chart 2 and Chart 3 show the comparison of clinical parameters before and after non surgical periodontal therapy in Group I- Chronic periodontitis patients. Wilcoxon signed rank test was used. The mean value for plaque index before and after non surgical periodontal therapy are 1.78 and 1.02 respectively. The significant p-value of this wilcoxon signed rank test infers that plaque level was reduced after therapy. The mean for gingival index before and after non surgical periodontal therapy are 2.1 and 1.46 respectively. The significant p-value infers that gingival inflammation was reduced after non surgical periodontal therapy. The mean value for probing depth before and after non surgical periodontal therapy are 3.19 and 2.55 respectively. The mean value for clinical attachment level before and after non surgical periodontal therapy are 3.21 and 1.87 respectively. Both the clinical parameters has significant p-value. This infers that non surgical periodontal therapy has reduced the probing depth and improved clinical attachment level.

Table No 2,Chart 4,Chart 5 show the comparison of clinical parameters before and after non surgical periodontal therapy in Group II- Chronic periodontitis patients with type 2 diabetes mellitus. Wilcoxon signed rank test was used. The mean value for plaque index before and after non surgical periodontal therapy are 1.97 and 1.06 respectively. The significant p-value of this wilcoxon signed rank test infers that plaque accumulation was reduced after therapy. The mean for gingival index before and after non surgical periodontal therapy are 2.34 and 1.61 respectively. The significant p-value infers that gingival inflammation was reduced after non surgical periodontal therapy. The mean value for probing depth before and after non surgical periodontal therapy are 3.18 and 2.66 respectively. The mean value for clinical attachment level before and after non surgical periodontal therapy are 3.23 and 2.15 respectively. Both the clinical parameters has significant p-value. This infers that non surgical periodontal therapy has reduced the probing depth and improved clinical attachment level.

Table 3 and Chart 6 show the comparison of baseline and post operative resistin level in Group I- Chronic periodontitis patients. Wilcoxon signed rank test was used. The significant p-value infers that non surgical periodontal therapy has reduced the levels of resistin comparing to it baseline level in chronic periodontitis patients.

Table 4 and Chart 7 show the comparison of baseline and post operative resistin level in Group II- Chronic periodontitis patients with type 2 diabetes mellitus. Wilcoxon signed rank test was used. The significant p-value infers that non surgical periodontal therapy has reduced the levels of resistin comparing to it baseline level in chronic periodontitis patients with type 2 diabetes mellitus.

Table 5, Chart 8 show the comparison of resistin level at baseline between two groups, Group I - Chronic periodontitis patients and Group II - Chronic periodontitis patients with type 2 diabetes mellitus. Mann Whitney U test was used. The mean values of resistin level for two groups at baseline are 62.44 and 59.09.The insignificant p-value infers that there is no statistical difference in resistin levels at baseline between two groups.

Table 6, Chart 9 show the comparison of resistin level after SRP between two groups, Group I - Chronic periodontitis patients and Group II - Chronic periodontitis patients with type 2 diabetes mellitus. Mann Whitney U test was used. The mean values of resistin level for two groups after SRP are 62.44 and 59.09.The insignificant p-value infers that there is no statistical difference in resistin levels after SRP between two groups.

TABLE NO 1: Comparison of clinical parameters before and after non surgical periodontal therapy (SRP) in Group I - Chronic periodontitis patients

Clinical parameters Chronic periodontitis patients	N	Mean	SD	Wilcoxon signed rank test p value
Plaque index at baseline	20	1.78	0.561	0.001*
Plaque index after SRP	20	1.02	0.426	
Gingival index at baseline	20	2.1	0.354	0.001*
Gingival index after SRP	20	1.46	0.37	
Probing depth at baseline	20	3.19	0.373	0.001*
Probing depth after SRP	20	2.55	0.349	
Clinical attachment level at baseline	20	3.21	0.711	0.001*
Clinical attachment level after SRP	20	1.87	0.356	

Chart 2: Comparison of Plaque Index and Gingival Index before and after SRP in Group I - Chronic periodontitis patients

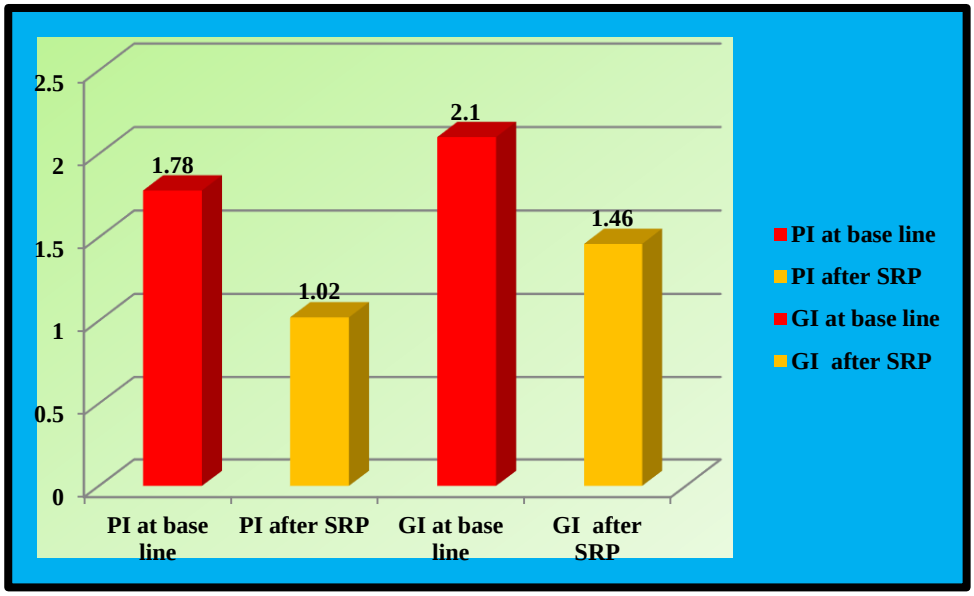


Chart 3: Comparison of Probing depth and Clinical attachment level before and after SRP in Group I - Chronic periodontitis patients

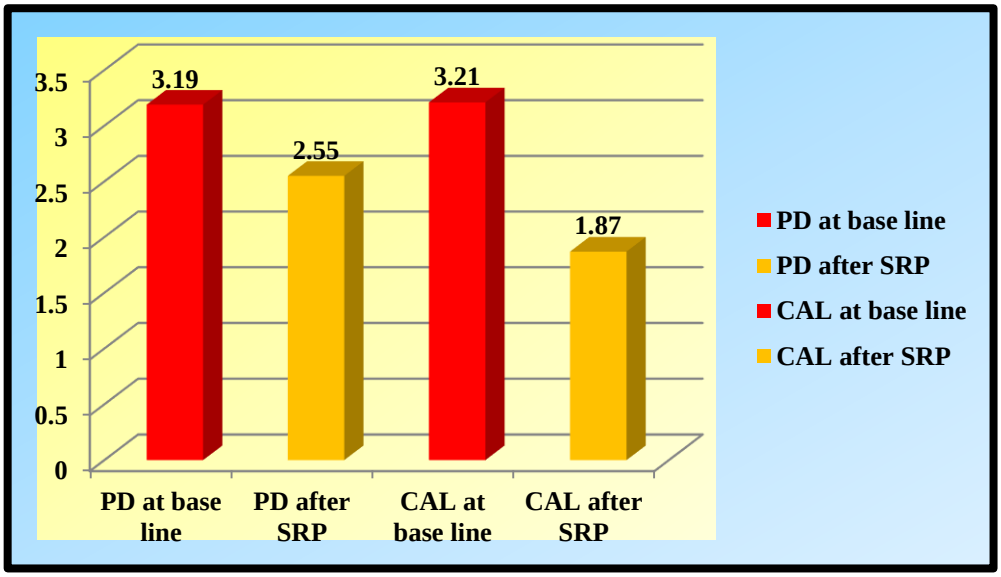


Table no 2: Comparison of clinical parameters before and after non surgical periodontal therapy (SRP) in Group I1- Chronic periodontitis patients with type 2 diabetes mellitus

Clinical parameters Group 2- Chronic periodontitis patients with type 2 diabetes mellitus	N	Mean	SD	Wilcoxon signed rank test p value
Plaque index at baseline	17	1.97	0.58	0.001*
Plaque index after SRP	17	1.06	0.28	
Gingival index at baseline	17	2.34	0.379	0.001*
Gingival index after SRP	17	1.61	0.291	
Probing depth at baseline	17	3.18	0.41	0.002*
Probing depth after SRP	17	2.66	0.241	
Clinical attachment level at baseline	17	3.23	0.663	0.001*
Clinical attachment level after SRP	17	2.15	0.38	

CHART 4: Comparison of Plaque index and Gingival index before and after SRP in Group II - Chronic periodontitis patients with type 2 diabetes mellitus

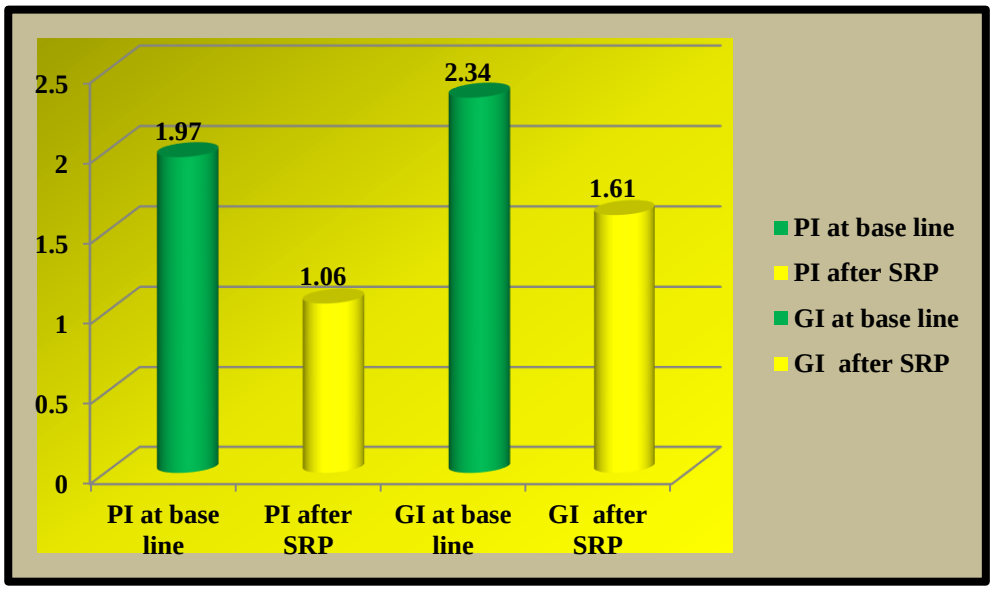


CHART 5: Comparison of Probing depth and Clinical attachment level before and after SRP in Group II - Chronic periodontitis patients with type 2 diabetes mellitus

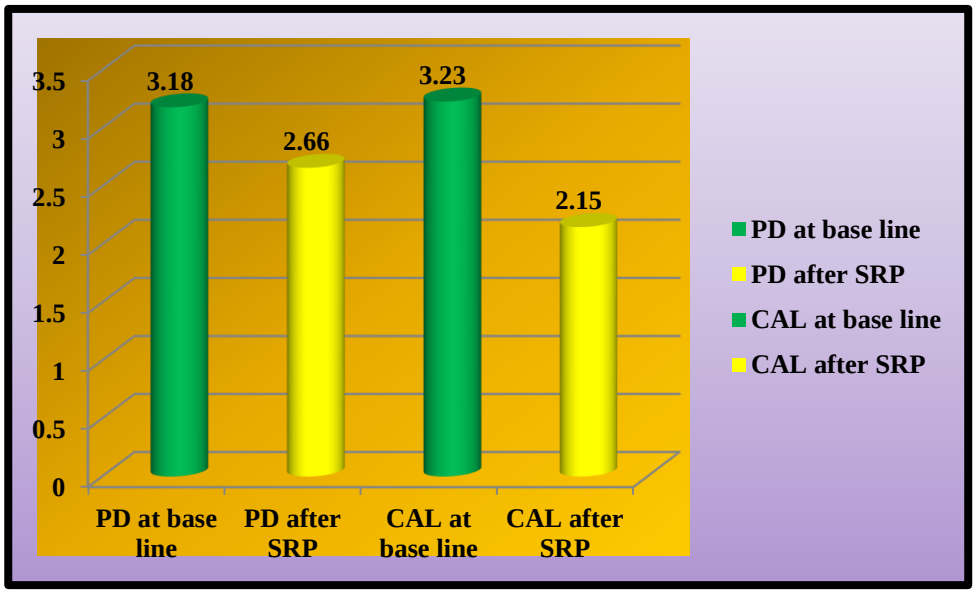


Table 3: Comparison of baseline and post operative resistin level in Group 1-Chronic periodontitis patients

RESISTIN LEVEL	N	Mean	SD	Wilcoxon signed rank test p value
Resistin level at baseline in Chronic periodontitis patients	20	62.44	11.577	0.001*
Resistin level after SRP in Chronic periodontitis patients	20	44.43	13.754	

CHART 6: Comparison of baseline and post operative resistin level in Group 1-Chronic Periodontitis patients

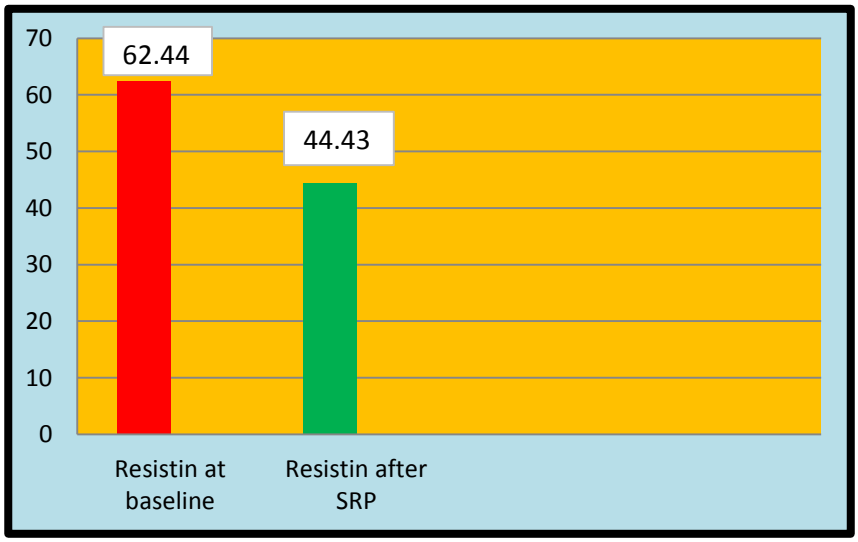


Table 4: Comparison of baseline and post operative resistin level in Group I1-Chronic periodontitis patients with type 2 diabetes mellitus

RESISTIN LEVEL	N	Mean	SD	Wilcoxon signed rank test p value
Resistin level at baseline in Chronic periodontitispatients with type 2 diabetes mellitus	17	59.09	7.062	0.001*
Resistin level after SRP in Chronic periodontitis patients with type 2 diabetes mellitus	17	42.36	7.057	

CHART 7: Comparison of baseline and post operative resistin level in Group II-Chronic periodontitis patients with type 2 diabetes mellitus

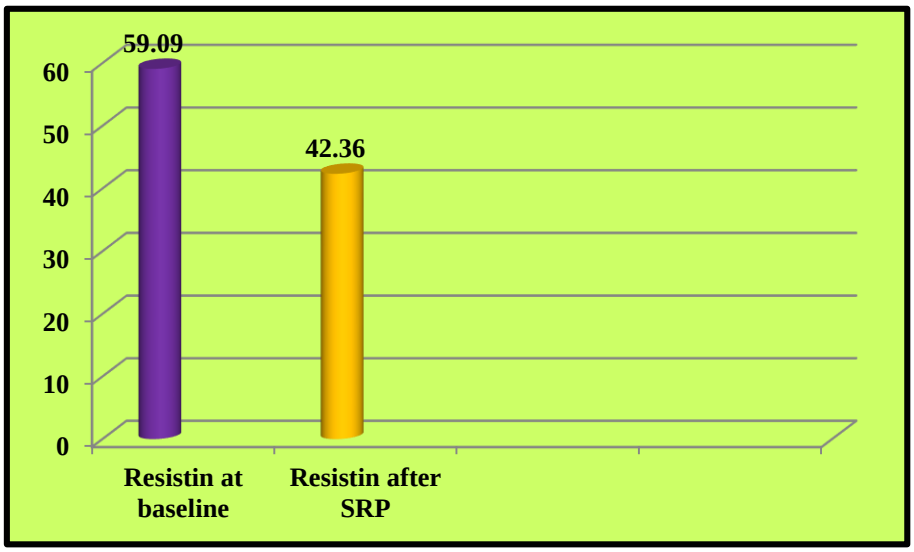


TABLE NO 5: Comparison of resistin level at baseline between two groups. Group I - Chronic periodontitis patients and Group II - Chronic periodontitis patients with type 2 diabetes mellitus

RESISTIN LEVEL		N	Mean	SD	Std. Error Mean	Mann whitney U test P value
RESISTIN at Baseline	Chronic periodontitis patients	20	62.44	11.577	2.589	0.201
	Chronic periodontitis patients with type 2 diabetes mellitus	17	59.09	7.062	1.713	

CHART 8: Comparison of resistin level at baseline between two groups. Group I - Chronic periodontitis patients and Group II - Chronic periodontitis patients with type 2 diabetes mellitus

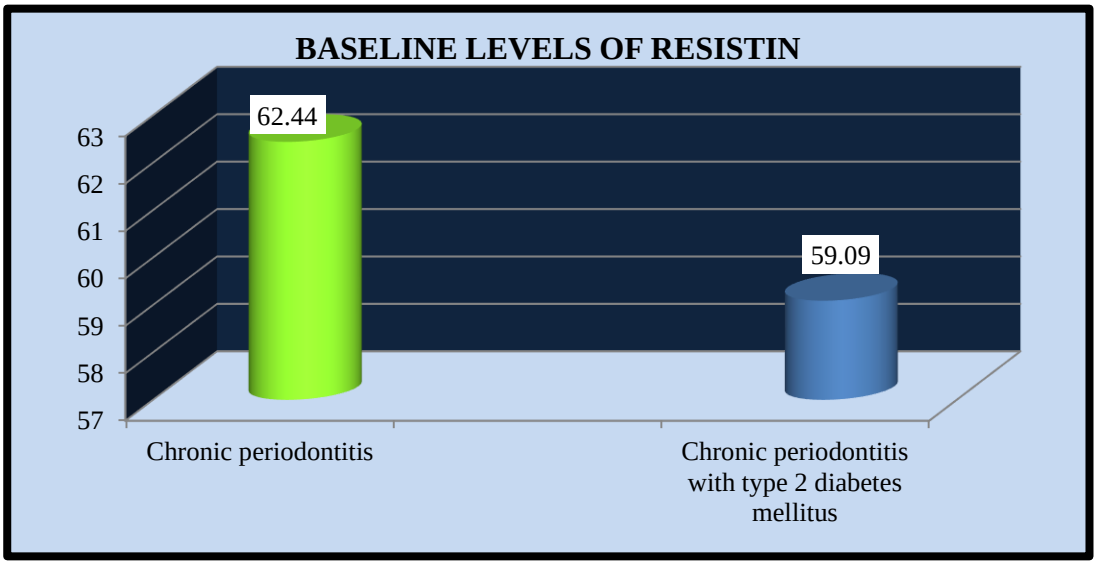
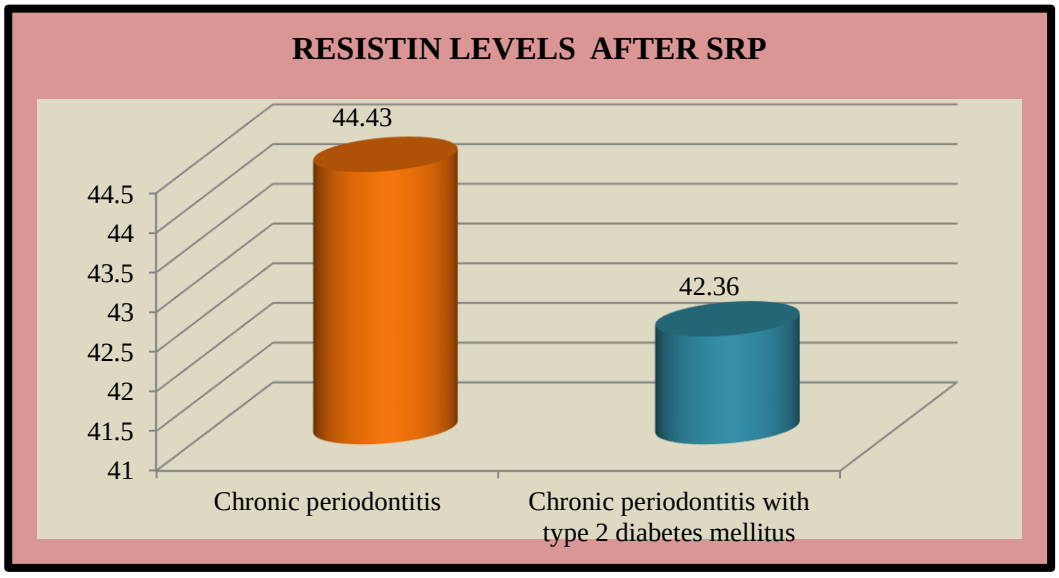


TABLE NO 6: Comparison of resistin level after SRP between two groups. Group I - Chronic periodontitis patients and Group II - Chronic periodontitis patients with type 2 diabetes mellitus

RESISTIN LEVEL		N	Mean	SD	Std. Error Mean	Mann whitney U test P value
RESISTIN after SRP	Chronic periodontitis patients	20	44.43	13.754	3.075	0.063
	Chronic periodontitis patients with type 2 diabetes mellitus	17	42.36	7.057	1.712	

CHART 9: Comparison of resistin level after SRP between two groups. Group I - Chronic periodontitis patients and Group II - Chronic periodontitis patients with type 2 diabetes mellitus



Chronic periodontitis is a result of chronic inflammatory response to the accumulated microbial plaque and calculus on the tooth surface. Toxic substances produce by microbes initiate the release of inflammatory mediators which results in host mediated destruction of periodontal tissue.[4]The presence of periodontal pathogens and the host response to pathogenic bacteria creates a complex bi-directional series of host-microbial interaction. As a result of this, cascade of events takes place.

During this cascade of events, various pro inflammatory cytokines and large quantities of destructive enzymes such as matrix metalloproteinase and inflammatory mediators are produced resulting in more extracellular degradation, osteoclast activation and differentiation, leading to further collagen and bone destruction ultimately ending up in more tissue destruction. Although the immune/inflammatory processes are essential for protecting the host against infection, the exaggerated host response to pathogenic bacteria results in periodontal tissue destruction.[5]

These inflammatory mediators also act as a biomarker for the periodontal disease. There are numerous biomarkers for periodontal disease, one such biomarker is the newly discovered Resistin. These are adipokines which are cysteine rich proteins, expressed in very low level in adipocytes and higher level in circulating monocytes, macrophages, lymphocytes and mononuclear leukocytes. These resistin are mainly involved in obesity and diabetes mellitus. Later, it was found to be involved in chronic periodontitis and various inflammatory conditions like rheumatoid arthritis, retinopathy, chronic kidney diseases, atherosclerosis, coronary artery diseases.[1,2,5]

Various immune cells like monocytes, macrophages, lymphocytes and mononuclear leukocytes act as a source for resistin in which mononuclear leucocytes (neutrophils) act as a major source of resistin. It acts as a proinflammatory molecule and stimulates the synthesis

and secretion of TNF-α and IL-12. Inturn these TNF-α, IL-6 and IL-1β increases the resistin expression in PBMCs, thus inducing its own production in a positive feedback cycle.[2] Lipopolysaccharides produced by periodontal pathogens also stimulates neutrophils and hence increased amount of resistin is released as an inflammatory exudate from GCF of inflamed periodontal tissues.[12]

Periodontitis being a sixth complication of diabetes, a bidirectional relationship has been established between diabetes and periodontitis. The interrelationship between diabetes mellitus and periodontitis has been studied for many years. At present there is strong evidence to suggest that the incidence and severity of periodontitis is influenced by the presence or absence of diabetes mellitus, as well as the degree to which the disease is controlled by patients.[32] As resistin levels are increased in periodontitis with chronic subclinical inflammation, the level of resistin increases in circulation and acts on GLUT. Thus it increases the insulin resistance by decreasing insulin sensitivity leading to increased risk for type II diabetes mellitus. Hence resistin could be one of the ideal biomarker which provides link among Resistin-Periodontitis-Diabetes mellitus triad.[1]

The aim of this study is to evaluate the levels of Resistin in gingival crevicular fluid before and after non surgical periodontal therapy in systemically healthy chronic periodontitis patients and in chronic periodontitis patients with Type 2 Diabetes mellitus. A total of 39 patients were included in the study and were divided into 2 groups – Group I (20 patients) : Chronic periodontitis patients and Group II (17 patients) : Chronic periodontitis with type II diabetes mellitus patients. There were 2 dropouts in Group II. So a total of 37 patients completed the study.

Clinical parameters like plaque index, gingival index, probing depth and clinical attachment level were measured at baseline and 3 months following non surgical periodontal

therapy. Plaque index helps in assessing the oral hygiene of the patients and gingival index aids in assessing the severity of gingival inflammation before and after SRP.[30] Patients with poor oral hygiene and severe gingival inflammation at 3 months after SRP were excluded from the study. Probing pocket depth and clinical attachment level were measured, as these are most important clinical parameters to evaluate the amount of clinical attachment loss or gain after SRP in chronic periodontitis patients. Hence probing depth ≥5mm was considered as defining criteria for chronic periodontitis.[2]Though bleeding on probing is the most important clinical sign which reflects the level of periodontal inflammation, it was not evaluated. And also the periodontal probe which was used is not pressure-calibrated to standardize probing forces.

In the present study, a period of 3 months has been chosen for re-evaluation after non surgical periodontal therapy for the assessment of both clinical parameters and levels of resistin. This is because, the appropriate time interval required for evaluating the effect of nonsurgical periodontal therapy which aids in complete resolution of gingival inflammation and tissue repair is approximately 3 months.[33] This resolution of the inflammation inturn reduces the level of resistin and hence biochemical analysis was also done after a period of 3 months following SRP.

GCF is an important diagnostic aid containing host cell products (cytokines, antibody, enzymes), products of tissue destruction, plasma derived molecules and subgingival microbial products.[2,14] Hiroshima et al (2012)[12] evaluated the levels of resistin in GCF, serum and synovial fluid and found mean resistin concentration in GCF was remarkably higher than serum and synovial fluid. As GCF concentration of resistin was higher than blood, GCF was chosen as a diagnostic aid for resistin. GCF derived biomarkers combined with clinical measures provide a greater specificity to identify the status of periodontal tissues and response to therapy. There are no previous studies in accordance with the present study. This

is the first study to evaluate the resistin levels in GCF of chronic periodontitis patients with and without type II diabetes mellitus before and after non surgical periodontal therapy.

There are few cross sectional studies which evaluated the levels of resistin in GCF of chronic periodontitis patients. These includes studies by Gokhale et al (2014)[2], Hiroshima et al (2012)[12], Suresh et al (2016)[28], Longo et al (2017)[29], and Mittal et al (2015)[26]. Serum levels of resistin was evaluated by Zimmerman et al (2013)[23], Rao et al (2016)[27] and Devanoorkar et al(2012)[21]. A study done by Devanoorkar et al (2012)[21] assessed the effectiveness of non surgical periodontal therapy on serum resistin levels and clinical parameters after SRP in chronic periodontitis patients.

The results of the study comparing the clinical parameters of both the groups, 3 months following non surgical periodontal therapy showed a statistical significant improvement. The positive clinical outcomes like reduction in probing depth and improvement in clinical attachment level obtained in both the groups are in agreement with the previously reported findings of Badersten et al (1987)[34] and Weijden et al (2002)[35] on the clinical efficacy of SRP in the treatment of chronic periodontitis. This study is another testament for the importance of nonsurgical periodontal therapy by means of SRP.

The results also showed statistically significant reduction in resistin levels in both the groups after non surgical periodontal therapy. As SRP reduces the subgingival load of periodontopathogens responsible for periodontal inflammation and tissue breakdown, there is reduction in inflammation. This in turn reduces the proinflammatory cytokines, which infers reduction in resistin level after SRP. This is the first study to evaluate the GCF resistin levels in chronic periodontitis patients after SRP. Similar to the present study, Devanoorkar et al (2012)[21] evaluated the resistin levels in chronic periodontitis patients after non surgical periodontal therapy (SRP). But he analysed in serum and obtained a negative correlation of

serum resistin levels after non surgical periodontal therapy. As mentioned previously, study by Hiroshima et al (2012)[12] showed concentration of resistin in GCF is higher than serum and hence GCF was used as a diagnostic aid in the present study which showed statistically significant levels of resistin after SRP.

On intergroup comparison of resistin levels at baseline and also after SRP, there is no significant difference in GCF resistin levels in chronic periodontitis patients and in chronic periodontitis patients with type 2 diabetes mellitus. There is no correlation of resistin levels at baseline which is in accordance with the study by Hiroshima et al (2012)[12], in which he concluded that there is no statistical difference in GCF resistin levels in chronic periodontitis and chronic periodontitis patients with type 2 diabetes mellitus. Contrary to our result, a cross sectional study by Gokhale et al (2014)[2] reported that GCF resistin levels were higher in chronic periodontitis patients with type 2 diabetes mellitus than in systemically healthy chronic periodontitis patients.

Certain limitations of the study pertaining to this result should be noted. Healthy group was not included in the study which fails to compare and correlate the clinical parameters with the resistin level in healthy and disease conditions. And also blood glucose level was not evaluated after 3 months following non surgical periodontal therapy. The reduced blood glucose level systemically may also reduce the resistin level which can also be the reason for insignificant resistin levels between two groups after non surgical periodontal therapy.

In brief, the limitations of the study are, healthy group was not included, blood glucose levels were not evaluated 3 months after SRP and pressure sensitive probe was not used to standardise the probing force.

Further clinical trials have to be done with proper study design and protocols, to determine the efficacy of non surgical periodontal therapy on resistin levels and periodontal disease condition.

Following conclusions were elucidated from the results of the present study

- Non surgical periodontal therapy – SRP is found to be effective in the treatment of chronic periodontitis patients with and without diabetes mellitus.
- The elevated levels of GCF resistin reflect the severity of periodontal disease.
- GCF resistin can be a useful biomarker to detect the periodontal disease condition.

Based on the results obtained from the present study, we summarise that GCF resistin is one of the novel biomarkers for periodontal disease and is also related to periodontal disease severity. Both the clinical parameters and resistin levels have significantly improved after non surgical periodontal therapy. Thus nonsurgical periodontal therapy may be regarded as an effective treatment approach for chronic periodontitis patients with and without diabetes mellitus.

Considering the limitations of the study, further studies on GCF resistin are needed with proper study protocols to draw more conclusive results.

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ANNEXURE 1

INFORMATION SHEET

We are conducting a study on "**EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON LEVELS OF RESISTIN IN GCF OF PATIENTS WITH CHRONIC PERIODONTITIS AND IN CHRONIC PERIODONTITIS PATIENTS WITH TYPE 2 DIABETES MELLITUS**".

The identity of the patients participating in the research will be kept confidentialthroughout the study. In the event of any publication or presentation resulting from the research, no personally identifiable information will be shared.

Taking part in the study is voluntary. You are free to decide whether to participate in the study or to withdraw at any time; your decision will not result in any loss of benefits to which you are otherwise entitled.

The results of the special study may be intimated to you at the end of the study period or during the study if anything is found abnormal which may aid in the management or treatment.

Name of the patient Signature / Thumb impression

Name of the investigator Signature

Date

ANNEXURE 2

INFORMED CONSENT FORM

EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON LEVELS OF RESISTIN IN GCF OF PATIENTS WITH CHRONIC PERIODONTITIS AND IN CHRONIC PERIODONTITIS PATIENTS WITH TYPE 2 DIABETES MELLITUS

Name: Age/Sex Op.No: Date:

Address:

I, _____ aged _____ have been informed about my role in the study.

1. I agree to give my personal details like name, age, sex, address, previous dental history & the details required for the study to the best of my knowledge.

2. I will co-operate with the dentist for my intra oral examination & extra oral examination.

3. I will follow the instructions given to me by the doctor during study.

4. I permit the dentist to take photos & I accept to undergo the procedures that are required for the study.

5. If unable to participate in the study for reasons unknown, I can withdraw from the study.

In my full consciousness & presence of mind, after understanding all the procedures in my own language, I am willing & give my consent to participate in this study.

Name of the patient: Name of the investigator:

Signature/Thumb impression Signature

ஆராய்ச்சி ஒப்புதல் கடிதம்.

பெயர் :- வயது/பாலினம் :- புறநோயாளி எண் :-

விலாசம் :- தேதி :-

திரு/திருமதி..... (வயது), ஆகிய நான் கீழ் காணப்படும் நிபந்தனைகளுக்கு ஒப்புதல் அளிக்கிறேன்.

1) என் பெயர், வயது, பாலினம், முகவரி, பல் சம்பந்தப்பட்ட சிகிச்சை மற்றும் என்னுடைய முழு விபரத்தினைக் கொடுக்க நான் முழு மனதுடன் ஒப்புக் கொள்கிறேன்.

2) என்னுடைய வாயின் உள்பகுதி (அல்லது) வெளிப்பகுதியை மருத்துவர் சோதனை செய்ய ஒத்துழைக்கிறேன்.

3) நான் மருத்துவர் அளிக்கும் விதிமுறைகளை தவறாமல் கடைபிடிப்பேன்.

4) மேற்கண்ட ஆராய்ச்சிக்கான என் புகைப்படம், உமிழ்நீர் மாதிரி மற்றும் பற்கள் சம்பந்தப்பட்ட எக்ஸ்ரே எடுக்கவும், ஈறு அறுவை சிகிச்சை செய்யவும் மருத்துவருக்கு ஒப்புதல் அளிக்கிறேன்.

5) நான் மேற்கண்ட ஆராய்ச்சிசியில் பங்கு பெற முடியவில்லை என்றால், ஆராய்ச்சியில் இருந்து விலகிக் கொள்வேன்.

மருத்துவரின் ஆராய்ச்சி சம்பந்தப்பட்ட விவரங்களை முழுமையாக புரிந்து கொண்டு பிறகு, என் முழுமனதுடனும் மற்றும் சுயநினைவுடனும் இந்த மருத்துவ ஆராய்ச்சியில் பங்குபெற சம்மதிக்கிறேன்.

நோயாளியின் பெயர் :- கையொப்பம் / பெருவிரல் ரேகை.

ஆராய்ச்சியாளரின் பெயர் :- கையொப்பம் :-

ANNEXURE 3

EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON LEVELS OF RESISTIN IN GCF OF PATIENTS WITH CHRONIC PERIODONTITIS AND IN CHRONIC PERIODONTITIS PATIENTS WITH TYPE 2 DIABETES MELLITUS

PROFORMA

S.No:

Name: O.P.No:

Age/Gender: Date:

Occupation:

Address and contact no:

Chief complaints:

Medical history:

Dental history:

Personal history :

Family history:

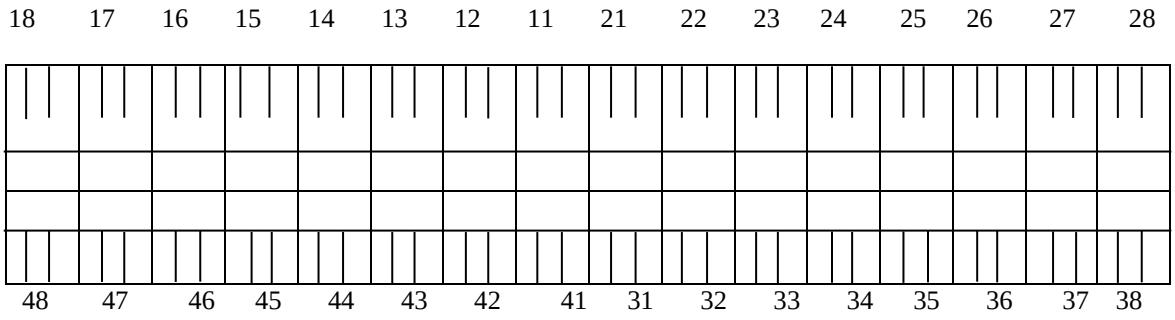
Oral hygiene measures :

Laboratory investigations :

Blood sugar level (RBS)	

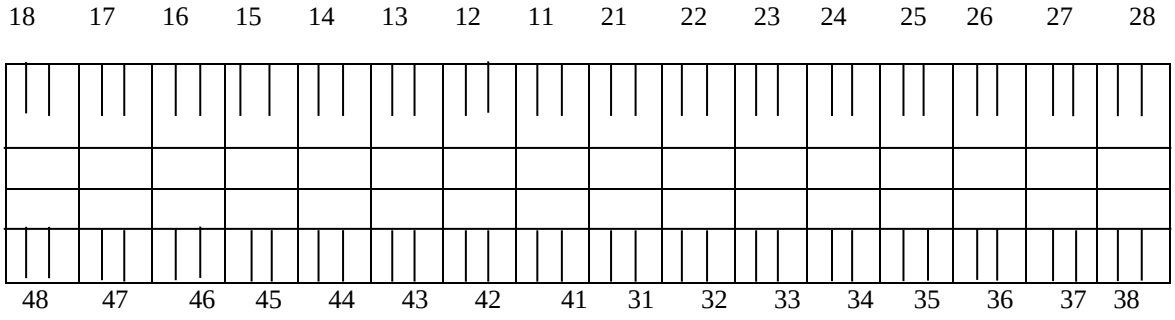
CLINICAL PARAMETERS AT BASELINE

PLAQUE INDEX – MODIFIED BY LOE (1967)



PI score=

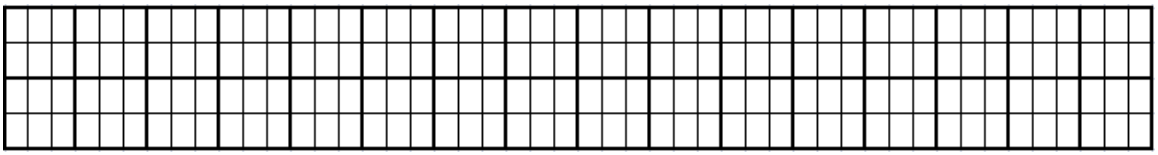
GINGIVAL INDEX – MODIFIED BY LOE in 1967



GI score=

PROBING POCKET DEPTH (in mm)

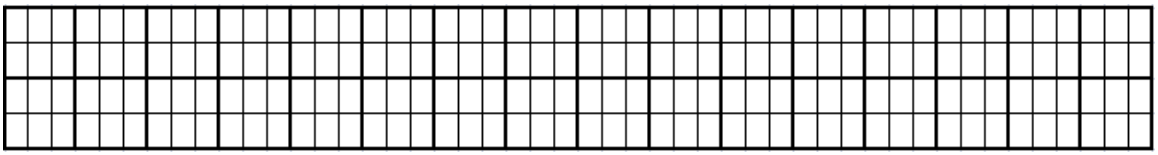
18 17 16 15 14 13 12 11 21 22 23 24 25 26 27 28



48 47 46 45 44 43 42 41 31 32 33 34 35 36 37 38

CLINICAL ATTACHMENT LEVEL (in mm)

18 17 16 15 14 13 12 11 21 22 23 24 25 26 27 28



48 47 46 45 44 43 42 41 31 32 33 34 35 36 37 38

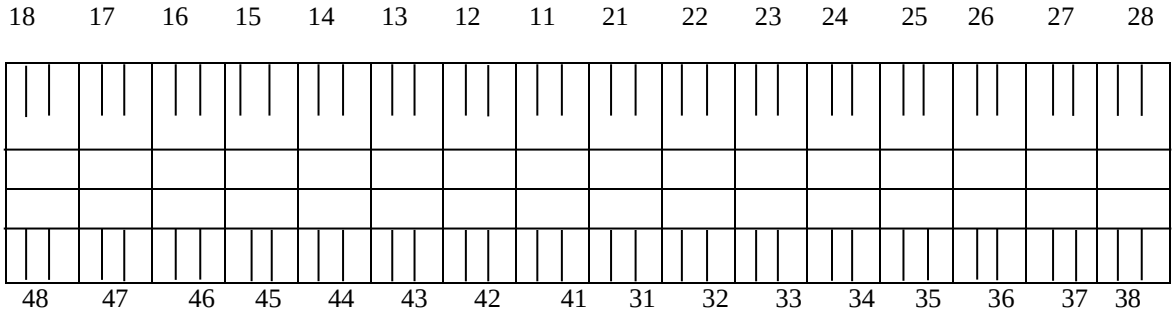
GCF SAMPLE COLLECTION:

TOOTH NUMBER	RESISTIN

SIGNATURE OF THE GUIDE

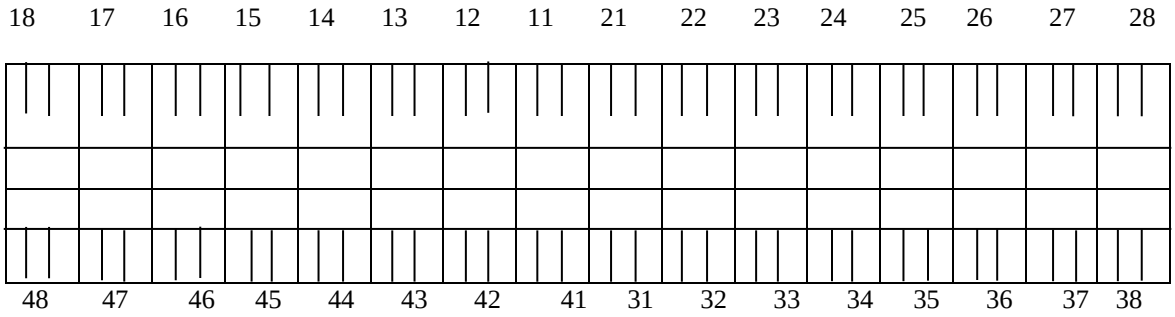
CLINICAL PARAMETERS AT 3 MONTHS (After SRP)

PLAQUE INDEX – MODIFIED BY LOE (1967)



PI score=

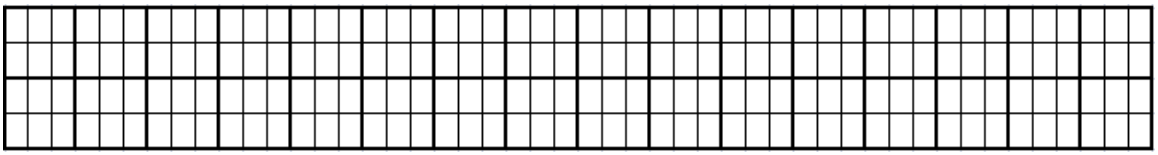
GINGIVAL INDEX – MODIFIED BY LOE in 1967



GI score=

PROBING POCKET DEPTH (in mm)

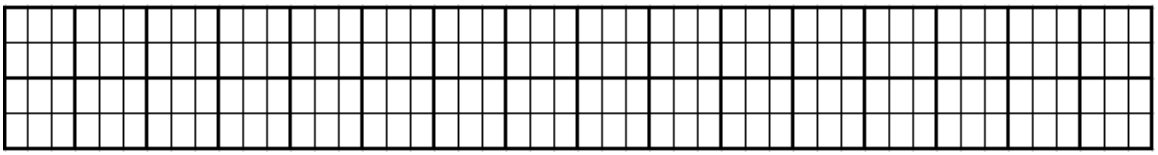
18 17 16 15 14 13 12 11 21 22 23 24 25 26 27 28



48 47 46 45 44 43 42 41 31 32 33 34 35 36 37 38

CLINICAL ATTACHMENT LEVEL (in mm)

18 17 16 15 14 13 12 11 21 22 23 24 25 26 27 28



48 47 46 45 44 43 42 41 31 32 33 34 35 36 37 38

GCF SAMPLE COLLECTION:

TOOTH NUMBER	RESISTIN

SIGNATURE OF THE GUIDE

INSTITUTIONAL ETHICAL COMMITTEE

KSR INSTITUTE OF DENTAL SCIENCE & RESEARCH

KSR Kalvi Nagar, Tiruchengode-637 215, Tamilnadu.
Phone : 04288-274981, Fax : 04288-274761,
email : ksrdentalcollege@yahoo.com

Ref.: 112 /KSRIDSR/EC/2015 **Date : 19.12.2015**

To

Dr.A.Tharani,
Postgraduate Student,
Dept. of Periodontics,
KSR Institute of Dental Science & Research,

*****

Your dissertational study titled "EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON LEVELS OF RESISTIN IN GCF OF PATIENTS WITH CHRONIC PERIODONTITIS AND IN CHRONIC PERIODONTITIS PATIENTS WITH TYPE 2 DIABETES MELLITUS" presented before the ethical committee on 15th Dec. 2015 has been discussed by the committee members and has been approved.

You are requested to adhere to the ICMR guidelines on Biomedical Research and follow good clinical practice. You are requested to inform the progress of work from time to time and submit a final report on the completion of study.

Signature of Member Secretary
(Dr.G.S.Kumar)

PLAGIARISM REPORT

URKUND

Urkund Analysis Result

Analysed Document:	PLAGIARISM.docx (D34250484)
Submitted:	12/30/2017 8:16:00 AM
Submitted By:	dratharani17@gmail.com
Significance:	2 %

Sources included in the report:

thesis print final.docx (D34109367)
plagiarism.docx (D30694268)
Shital A. Hungund Ch-3.pdf (D20654568)

Instances where selected sources appear:

6

CERTIFICATE

This is to certify that this dissertation work titled "**EFFECT OF NON SURGICAL PERIODONTAL THERAPY ON LEVELS OF RESISTIN IN GCF OF PATIENTS WITH CHRONIC PERIODONTITIS AND IN CHRONIC PERIODONTITIS PATIENTS WITH TYPE 2 DIABETES MELLITUS"** of the candidate **A.THARANI** with registration number **241513253** for the award of **MASTER OF DENTAL SURGERY** in branch II **PERIODONTOLOGY.** I personally verified the urkund.com for the purpose of plagiarism check. I found that the uploaded thesis file contains from introduction to conclusion pages and result shows **2** percentage of plagiarism in the dissertation.

Guide and Supervisor sign with seal